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SEP 75 B S SAVARA, R W FIELDS, R B TACKE DADA17-72-C-2185

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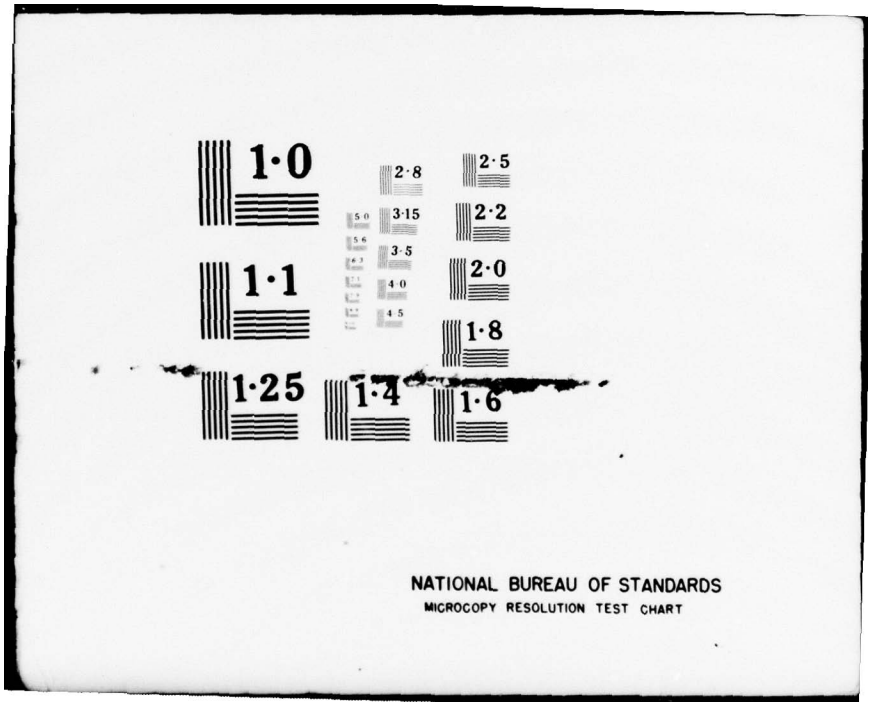
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ELECTRICAL ENHANCEMENT OF HEALING IN COMBAT INJURIES TO HARD AND SOFT TISSUES

ANNUAL SUMMARY REPORT

B. S. SAVARA, D.M.D., M.S.
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R. B. TACKE, B.S.
M. H. BARTLEY, D.M.D., Ph.D.

September 30, 1975

Supported By

DENTAL RESEARCH BRANCH
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AND DEVELOPMENT COMMAND
Washington, D.C. 20315

Contract No. DADA-17-72-C-2185

Biophysics Laboratory
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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*
This document reports the results of experiments designed to determine the effects of the electrical stimulation of circumscribed defects in the dog calvarium. Comparisons of the effects of current level, current type, polarity and the temporal period of stimulation are included.

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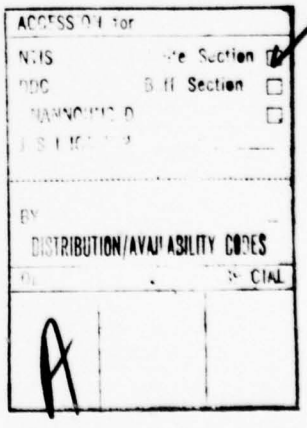

ABSTRACT

Previous communications from this laboratory have established that exogenous electrical currents enhance the healing of bone defects. In addition they have indicated which are the most propitious times to apply current to healing wounds. Also indicated in these reports were what electrode positions were most effective.

The present series of experiments extended the initial work and further pinpointed the optimal times for current application. The effects of wave form were elucidated in the recently-completed series as well. Differences were noted in the pattern of enhancement brought about by alternating current as opposed to direct currents.

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INTRODUCTION

Bioelectricity is found to be of great importance in the control of numerous processes among which are such diverse phenomena as: stimulation of the synthesis of macromolecules, limb regeneration, bone wound healing, modeling of bone, thrombosis formation, and cell membrane functions (1, 2, 6, 7, 8, 9). This listing is by no means inclusive. Our concern here is with the influence which electrical currents have on healing bone wounds. A number of reports demonstrate electric potentials present in mammalian bone *in vivo*. Bones, when subjected to mechanical stresses, exhibit minute electric potentials in precise geometrical correlation to the mechanical forces of deformation. Another class of potentials, the static or standing potentials, also exists associated, it is thought, with life processes of the cellular populations of the bone tissue (6, 10).

The above observations have provided impetus to many investigators involved in the study of both physiology and the healing of wounds to apply currents, both alternating and direct, using a wide range of current density to a variety of wounds (11-18). Varying degrees of success have been reported based upon classical biological and clinical endpoints which include "breaking strength", "bending strength", (tensile and compression strength), radiographic evaluation of the resultant callous and histological examination in hard tissue, the tensile strength of the so-called scar, gross inspection and microscopic examination of the scar in soft tissue. These biological endpoints are of undisputed value but yield qualitative or, at best, semi-quantitative results which do not furnish a direct answer to the question of whether or not the application of current does accelerate or enhance bone apposition. Further, it is reasonable to assume that without collection of quantitative data that can be subjected to statistical analysis the understanding of the mechanism by which acceleration or enhancement of the reparative process resulting from the application of electrical current occurs will be impossible.

Our focus of study is the quantitative demonstration of exogenous electric current enhancement of bone wound healing. After it was established that accelerated healing occurred, it became manifestly important to find out what conditions of employment would optimize the effects of exogenous currents. Time of application, wave forms and electrode configuration were evaluated quantitatively and qualitatively *in vivo* and *in vitro*.

ELECTRICAL CURRENTS IN HARD TISSUE REPAIR *IN VIVO*

GENERAL APPROACH

Over the past decade a large body of experimental evidence has accumulated showing the positive influence of electric currents on bone metabolism (19-26). It is evident that modeling, remodeling and repair are regulated through bone's piezoelectric properties as mechanical transducers in a closed loop system (1, 2, 7). In situations where enhancement of normal function of the transducer system is desirable, viz during bone repair, it would be advantageous to use an exogenous source of electric current.

One must keep in mind that there is a direct positive correlation in a given bone or portion thereof between the amount of calcified tissue (as determined by bone ash values) and the compression or "breaking strength" (27-28). It follows from this that the degree of bone healing is also directly related to the quantity of bone being formed in the wound. The rate of bone production can be directly determined by tetracycline labeling.

In previous annual reports we have shown that the application of exogenous currents accelerates the repair of wounds in bone. In addition these reports have yielded data suggesting what conditions might be more efficacious. Our present report extends these previous observations and establishes them quantitatively. Questions regarding the relative advantages or disadvantages of electrode configuration, wave forms and time of application were specifically dealt with in this series of experiments. The question posed in the previous annual report regarding possible mechanisms by which repair enhancement occurs has not as yet yielded to the quantitative techniques employed at present, but a proposal has been advanced (this year's contract renewal document) to elucidate qualitatively in a serial sacrifice study the histologic steps encountered in healing sites influenced by exogenous electrical currents. The quantitative data, however, will always supersede in biological importance the qualitative findings because the fundamental, practical questions to be answered are: "What are the rates at which bone forms during healing processes (normal and abnormal)?" and, further, "Does the electrical current supplied change these rates significantly?" A means of measuring bone accretion rates will always be necessary in order to validate any observed qualitative processes assumed to be instrumental in the acceleration of bone repair. Due caution must be exercised during the interpretation of qualitative studies not to confuse bone reparative processes with remodeling of bone.

We have continued, as in the past, to use radiographic and histologic data to monitor the progress of the experiment. Paralleling the quantitative bone accretion studies (with tetracycline labeling), rat in vivo and primate fibroblast tissue and cell culture in vitro studies were conducted to inspect certain aspects of current level, wave form and electrode materials as well as to expedite feedback of information to the experimentor.

Below is a detailed account of our activities from June 1974 to June 1975.

MATERIALS AND METHODS

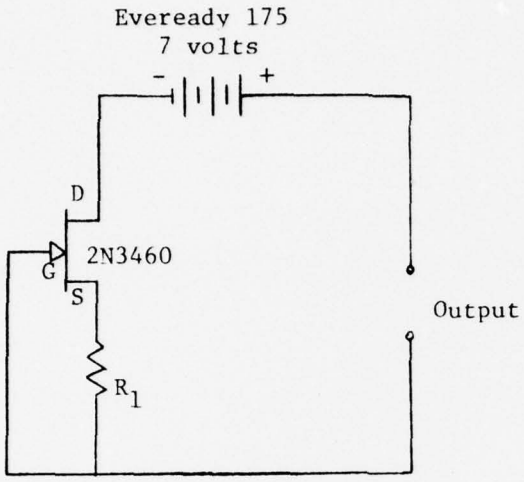
Thirty genetically-defined dogs, 10-12 months of age, weighing 25-35 lbs. total body weight were utilized in the in vivo quantitative measurement of calvarium defect repair. Under strict asceptic techniques with the animal in the prone position, general anesthetic was administered and the parietal bones were reached through a midline incision extending from the occipital protuberance along the length of the sagittal crest.

Fascia and muscles were separated by sharp dissection and retracted laterally on each side, exposing the parietal bones. Four circumscribed defects, 4 mm square, were cut through the cortical and spongy bone into the marrow of each bone using a dental handpiece with a concurrent flushing of the area with large volumes of physiological salt solution. Hemostasis was maintained in the defect using Gelfoam (29). Threaded stainless steel electrodes, 2 mm in diameter, with 34-gauge, Teflon-coated (8), stainless steel wire attached were (1) in the case of the balanced electrode system, implanted 8 mm from the center of each defect; and (2) in the case of the unbalanced electrode system, one electrode was implanted 4 mm from the center of the defect while the other was implanted 8 mm from the center of the defect (refer to Fig. I). These wires were brought from the electrode site through the muscle and fascia and finally exposed through a small skin incision on the dorsal aspect of the neck of the animal at the fourth cervical vertebra. Hemostatic material was removed, and the entire area was again rinsed with large volumes of saline to remove debris. Muscle and fascia were approximated and closed using absorbable, silk, interrupted sutures. A small constant current, alternating or pulsed direct current power supply (refer to Fig. I) was attached to the appropriate electrode pair, and the power unit with the additional electrode pairs was wrapped in a sterile dressing around the neck of the animal. This dressing was periodically removed and replaced to change electrode pairs and for routine inspection. Fully charged power sources are utilized for each healing interval. The current output of each power supply is measured prior to its use and upon its removal from the animal. In the rare instance of malfunction the data from the healing defect involved is considered unsuitable for analysis.

In the initial series of experiments, a constant direct current from a battery operated constant current power supply of 0.10 microampere was applied to a separate electrode pair for each of three consecutive 14-day healing intervals. This series was repeated using 1.0 and 10.0 microamperes. During the period of current administration, daily physical examination and weekly hematological and serum chemistry evaluations were reported on each animal to provide specific evidence of the time course of systemic indices of healing and to help identify individual abnormalities. Tetracycline hydrochloride (30), a label of bone apposition (29), was administered intravenously (10 mg/kg total body weight) at 1, 3 and 5 weeks postoperatively.

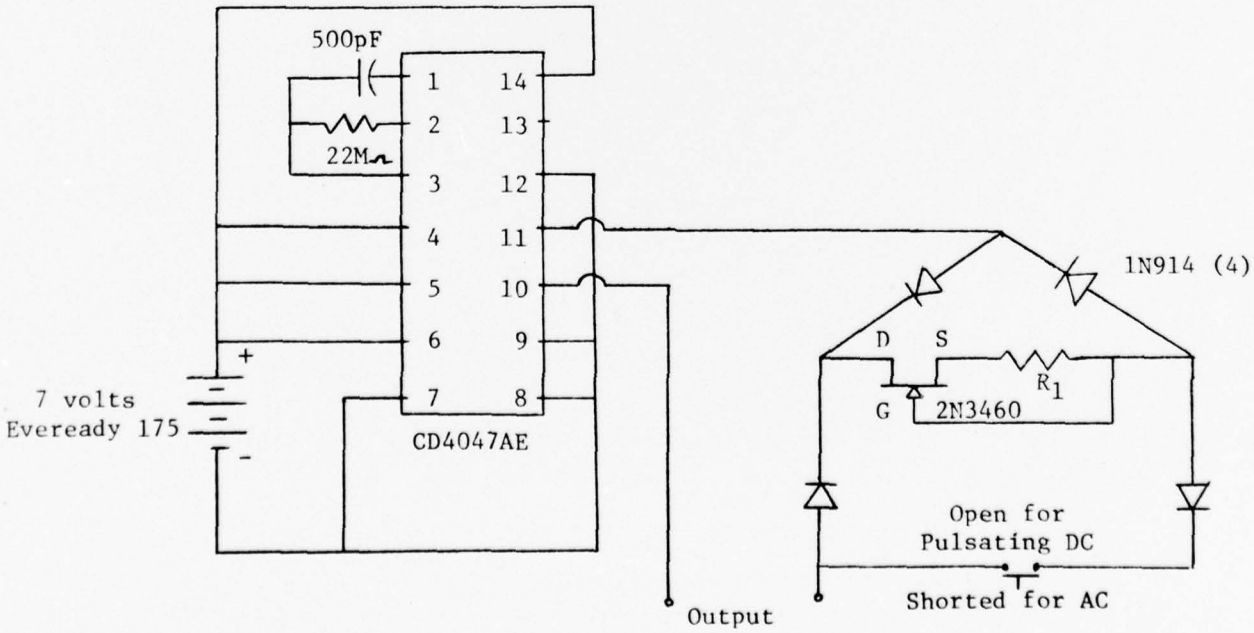
FIGURE 1

Direct Current Power Supply



R_1 - Selected to match individual 2N3460 to yield desired power output

Alternating and Pulsed Current Power Supply



R_1- Selected to match individual 2N3460 to yield desired power output

On completion of the experimental protocol, the 42nd postoperative day, the animal was sacrificed and a complete autopsy was performed, with special attention being given to all factors that are related to the reparative process. Parietal plates including the defects were removed, radiographed and fixed in acetone from which ground sections were prepared for tetracycline fluorescent labeling analysis of the appositional bone growth. The experimental series were designed to provide both histological examination and fluorescent analysis of two distinct specimens from each defect. Each animal affords a total of 8 defects as depicted in Fig. II. Four of these were chosen at random as the test specimens, and the remaining 4 were controls, prepared in exactly the same manner as the other 4; but the subsequent administration of current was deleted. Additional controls included animals with defects but lacking implanted electrodes in order to determine the potential effect of the presence of stainless steel electrodes, and animals having both defects and electrodes but lacking current administration at any site in order to evaluate the effects of current spread from a test defect to the control sites.

Analysis of 100-150 micron-thick, ground, undecalcified, calvarium sections was accomplished utilizing a Leitz fluorescent microscope and an eyepiece micrometer (Bausch and Lomb). The mean of 12 micrometer measurements was recorded for each defect, test and control (31).

Daily physical examinations including body temperature, pulse rate and a description of the general state of health were maintained on each animal throughout the experiment.

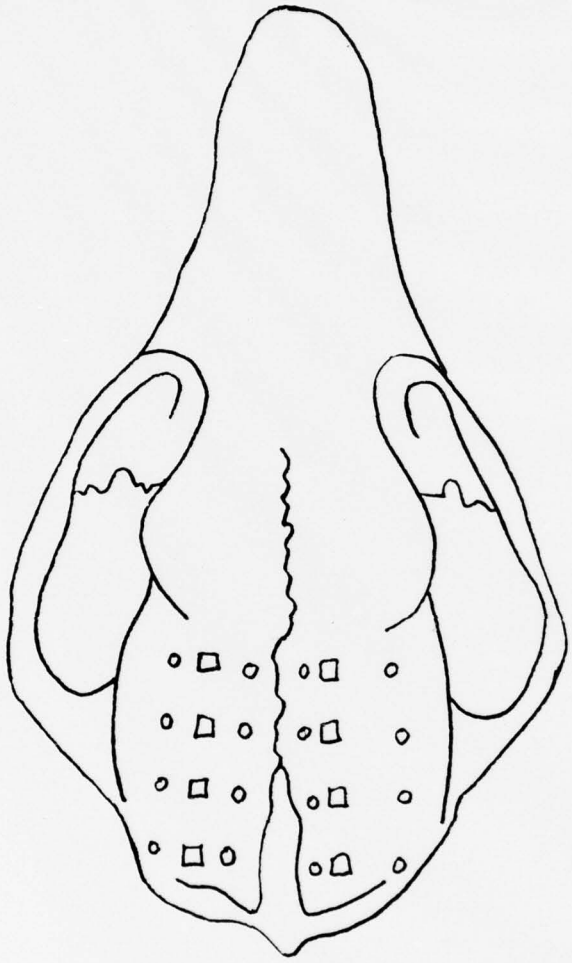
All animals showed a minor temperature elevation (less than 1° F.) for 1-2 days postoperatively with a return to normal by the third day. Pulse rate showed no significant change. All animals were described as appearing in either good or excellent health on the first postoperative day with the exception of three that showed mild to marked edema in the region of the head and neck. This was immediately alleviated by loosening the bandage around the neck of each animal to allow adequate venous return. These animals were then described as appearing in good or excellent health on the following morning.

RESULTS AND DISCUSSION

At sacrifice, all 30 animals were described as fully-developed, adult dogs in apparent good to excellent health, each showing a completely healed incision extending anterior from the occipital protuberance along the entire length of the sagittal crest.

The autopsies performed through a standard Y incision showed all organs of

FIGURE 2



Top view of dog cranium indicating the general arrangement of the eight bone defect-electrode pair complexes. Each complex consists of a central defect 4 mm square straddled by two remote electrodes. The left depicts the balanced electrode system and the right the unbalanced electrode system. The electrical circuit of each complex is completely isolated from the circuit of other complexes and from ground, limiting the current flow between a particular electrode pair to a uniform density through the associated defect without spread to neighboring sites.

the thorax and abdominal cavities to appear within normal limits in the dogs examined. On gross examination, all the defects of the animals that had received current were in a more advanced state of repair than those animals that had not received current. Also by gross examination it appears that those defects having directly received current in the earlier stages of the reparative process show slightly more advanced repairs than both those receiving current in the later stages of repair and those defects in the same animal having received no current. This gross observation was confirmed by examination of the post-mortem radiographs.

Specimens from 30 dogs have been analyzed this year.

Three direct current electrode configurations have been examined at 3 different current levels. These studies were initiated in 1974, and completed during the current year, 1975. Data cells in the 1.0 microampere and 10 microampere levels under conditions of anode proximate and cathode proximate to the defect respectively were completed in the current, 1975 series of experiments. The data cells of the balanced electrode configuration at 10 microamperes were not done because it appeared from pilot studies that this level of stimulation would not produce the desired effects in the balanced configuration. The results of the aforementioned studies are summarized in Table I. We have extended the recent work to include comparisons of alternating, direct and pulsed direct current effectiveness in enhancing the rate of bone wound healing. These comparisons have been made at two current levels, 1.0 microampere and 0.1 microampere. These findings are presented in Table II. The effect of time of application has been refined and data compared from three time intervals (1-2 wk., 3-4 wk. and 5-6 wk.) utilizing direct, alternating and pulsed direct currents (Tables III and IV). Quantification of data from all the studies enumerated above is presented in units of bone accretion (microns) over the experimental period.

1. Effect of Electrode Configuration and Current Levels on the Rate of Bone Healing (Table I)

   Balanced electrode configurations at two current levels, 0.1 microampere and 1 microampere show an approximately two-fold increase in the amount of bone accretion during the period of experimental stimulation. Both current levels, furthermore, under these conditions are not significantly different in their ability to effect rate changes. Inspection of the table will also show a remarkable reversal of effects at the proximate anode between the 0.1 microampere level ($113 \pm 79\ \mu$) and the 10 microampere level ($1162 \pm 311\ \mu$). The cathode placement proximate to the defect showed an increase uniformly in accretion rates at the three current levels employed.

2. Effect of Alternating, Direct and Pulsed Direct Current on the Rate of Bone Healing (Table II)

TABLE I

Bone Accretion in Circumscribed Surgical Defects in the Dog Calvarium Under Three Different Current Levels of Electric Stimulation and Three Electrode Positions.*

	Control	0.1 uA	Control	1.0 uA	Control**	10 uA
Balanced Electrode Configuration	461 ± 333 μ	807 ± 193.25 μ t = 2.65 P 0.010 n = 20	475 ± 254 μ	865 ± 433 μ t = 5.30 P 0.005 n = 82	Not Done	
Anode Proximate to Defect	461 ± 333 μ	113 ± 79 μ t = 2.27 P 0.025 n = 17	475 ± 254 μ	518 ± 299 μ t = .460 P Not Sig. n = 65	481 ± 305 μ t = 5.09 P 0.005 n = 28	1162 ± 311 μ
Cathode Proximate to Defect	461 ± 333 μ	785 ± 354 μ t = 2.15 P 0.025 n = 21	475 ± 254 μ	1106 ± 682 μ t = 5.38 P 0.005 n = 67	481 ± 305 μ t = 3.11 P 0.005 n = 29	905 ± 370 μ

*Measurements in μ ± standard deviation
**Control values in the 10 uA cell are derived from animals not having received current.

TABLE II

1 Microampere Alternating Current vs. Control

$864 \pm 415\ \mu$ $470 \pm 218\ \mu$

$t = 7.410$
$P \leqq 0.001$
$n = 47$

1 Microampere Direct Current (Pulsed) vs. Control

$877 \pm 495\ \mu$ $470 \pm 218\ \mu$

$t = 3.778$
$P \leqq 0.001$
$n = 51$

1 Microampere Alternating Current vs. 1 Microampere Direct Current (Pulsed)

$864 \pm 415\ \mu$ $876 \pm 495\ \mu$

$t = 0.245$
$P \leqq$ Not Sig.
$n = 48$

1 Microampere Direct Current vs. 1 Microampere Direct Current (Pulsed)

$1106 \pm 682\ \mu$ $877 \pm 495\ \mu$

$t = 1.155$
$P \leqq$ Not Sig.
$n = 37$

1 Microampere Alternating Current vs. 1 Microampere Direct Current

$864 \pm 415\ \mu$ $1106 \pm 682\ \mu$

$t = 1.276$
$P \leqq$ Not Sig.
$n = 33$

TABLE III

Early vs. Late Application of Direct Current (1.0 Microampere) to Circumscribed Surgical Defects in the Dog Calvarium

TREATMENT PERIOD:	BONE ACCRETION DURING THE EXPERIMENTAL PERIOD:
Control	488 $\pm$ 289 μ
*Early Application	890 $\pm$ 254 μ
**Late Application	371 $\pm$ 222 μ

t Test Control vs. Early Application
n = 48, t = 4.54, Significant at $P \leqq 0.01$

t Test Control vs. Late Application
n = 48, t = 1.37, Not Significant

*Early application period = 1st 3-wk. period (Wks. 1-3)
**Late application period = 2nd 3-wk. period (Wks. 4-6)

Stated simply, all wave forms employed are capable of significant stimulation of bone defect healing. Individual comparisons of AC to DC, or AC to DC pulsed, or DC to DC pulsed failed to show significant differences between them.

3. Effect of Application Time and Wave Form on Bone Healing with Electric Currents (Tables III, IV and V)

In a previous report (Table III) we noted a significant enhancement of healing when an early time period (1-3 wks.) was chosen for current application versus normal rates when the current was applied in a later time period (4-6 wks.) following wounding. Our recently-completed studies (Tables IV and V) have demonstrated that applications at two early intervals, namely 1-2 wks. and 3-4 wks., show significant increases of bone accretion with direct (pulsed) current and alternating current. A fascinating additional finding is that when alternating current is employed in the 5-6 wk. interval, a significant enhancement of bone formation occurred. This finding is rather unexpected in view of previous work in this laboratory which showed only early periods of applications effective.

When examining the time periods (1-2, 3-4 and 5-6 wks.) compared to each other at 1 microampere direct (pulsed) current, it is evident that the two earlier time periods (1-2 and 3-4 wks.) do not show a particular advantage one over the other ($880 \pm 350\ \mu$ vs. $924 \pm 497\ \mu$), but they both yield a significant advantage over currents applied in the latest period (5-6 wks., $449 \pm 296\ \mu$).

In consideration of the data from the 1 microampere AC study, it is equally evident that enhanced healing patterns exist with all accretion rates in the three intervals elevated over controls. It is further evident that at the middle time period (3-4 wks.) healing rates are slightly more elevated though not to a significant degree over the first interval (1-2 wks.) but are significantly elevated over the third time period (5-6 wks.)

In five animals we created eight surgical defects but no electrodes nor current were utilized. In an additional five animals eight defects and their associated electrodes were placed but current was not applied. The bone apposition rate from these defects did not differ significantly from comparable control sites in animals which did receive current application in test sites.

All data reported in the tables were examined using a student to test with the knowledge that they failed to meet all of the assumptions required for parametric analysis. This method was utilized for convenience and it was assumed that the reported significance levels would be lower with its use than with the use of nonparametric analysis. Portions of the data were subsequently analyzed using nonparametric analysis and in all cases the level of significance proved to be more significant than when examined by parametric analysis.

TABLE IV

Comparison of Current Application at Varied Time Intervals During the Reparative Process

1 Microampere Direct Current (Pulsed)

Interval of Current Application Week (Post-Op)	1 - 2	3 - 4	5 - 6	Control
Bone Accretion in Microns	880 ± 350	924 ± 447	449 ± 296	410 ± 245

1-2 Wks. vs. Control	t = 3.349 P ≦ 0.005 n = 18
3-4 Wks. vs. Control	t = 3.360 P ≦ 0.005 n = 22
5-6 Wks. vs. Control	t = 0.294 P ≦ Not Sig. n = 13
1-2 Wks. vs. 3-4 Wks.	t = 0.223 P ≦ Not Sig. n = 18
1-2 Wks. vs. 5-6 Wks.	t = 2.376 P ≦ 0.05 n = 14
3-4 Wks. vs. 5-6 Wks.	t = 2.331 P ≦ 0.05 n = 18

TABLE V

Comparison of Current Application at Varied Time Intervals During the Reparative Process

1 Microampere Alternating Current

Interval of Current Application Week (Post-Op)	1 - 2	3 - 4	5 - 6	Control
Bone Accretion in Microns	840 ± 482	1262 ± 605	803 ± 371	473 ± 201

1-2 Wks. vs. Control — t = 2.149, P ≦ 0.05, n = 16

3-4 Wks. vs. Control — t = 3.801, P ≦ 0.005, n = 17

5-6 Wks. vs. Control — t = 2.437, P ≦ 0.025, n = 19

1-2 Wks. vs. 3-4 Wks. — t = 1.327, P ≦ Not Sig., n = 13

1-2 Wks. vs. 5-6 Wks. — t = 0.168, P ≦ Not Sig., n = 15

3-4 Wks. vs. 5-6 Wks. — t = 2.055, P ≦ 0.05, n = 16

ELECTRICAL CURRENTS IN SOFT TISSUE REPAIR IN VIVO

GENERAL APPROACH

The studies of soft tissue repair are analogous to the investigations in hard tissue. Certain aspects of the reparative processes are best studied in the former due to the greater ease with which specimens can be processed.

The model chosen for this experimental series was the abdomen of the Sprague Dawley rat for two reasons. First, the reparative process of this tissue has been described in detail in the literature (33-35), and secondly, the methods for the restraint of this animal during current administration have been previously worked out in our laboratory (32). These methods were employed during the past contract year simply to establish whether or not the lower current densities would produce an observable effect prior to its administration to the hard tissue series.

MATERIALS AND METHODS

Using aseptic techniques with the animal under general anesthesia in the supine position, two 1 cm long incisions were made through the skin and fascia to a depth of 5 mm into the muscles of the abdomen on either side of the midline. Stainless steel or platinum electrodes were implanted on both sides of each incision 6-8 mm from the center of the lesion, and the attached wire leads run subcutaneously to the exterior of the dorsal surface of the neck of the animal. With the animal restrained in a special cage and restraint system described in a previous communication (32), a pulsed direct current of 0.1 microampere was applied during week 1-3 of the reparative process of one of the two lesions on the abdomen of the animal, the remaining lesion serving as a control. This protocol was repeated in five animals. Similar protocols were pursued in five animals each using pulsed direct current at 1.0 microampere and alternating current at 0.1 and 1.0 microampere levels.

Histological examination utilizing a so-called blind technique in that the histologist, unaware of how the tissue had been treated, was asked to evaluate the sections on a 1+ to 4+ basis with regard to the state of repair (1+ designating the least advanced repair and 4+ designating the most advanced repair). In the examination of the tissue from the electrode sites, he was asked to designate by number the appearance of the section again on a 1+ to 4+ basis, 4+ designating normal microscopic anatomy and 1+ designating advance inflammatory response or definite tissue destruction. In the examination of those sections stained for examination of collagen (trichome stain), the histologist

was asked to estimate both the quantity of collagen and quality of collagen bundles on the basis of 1+ to 4+, the lowest (1+) value designating the least amount and/or lowest quality, and the highest value (4+) designating the greatest amount and/or highest quality collagen.

RESULTS AND DISCUSSION

The results in the soft tissue correspond to those reported in the hard tissue section of this document. All lesions receiving current during the first 3 weeks of the reparative process were evaluated as being in a state of more advanced repair than were their respective controls. These evaluations were made based upon increased vascularization and a decreased inflammatory infiltrate in the lesions receiving current.

No attempt to date has been made to evaluate the tissue proximate to the electrode sites or to compare the individual wave forms or current densities.

ELECTRICAL CURRENTS IN CELL AND TISSUE CULTURES IN VITRO

GENERAL APPROACH

One must conclude that the application of energy to any biological system that results in an alteration of that system must effect changes at the cellular level and probably at the organelle level or below. In order to understand and better utilize the mechanisms of action of electrical energy on biological tissue, we must endeavor to determine the influence of this energy at the cellular level.

The initial in vitro studies have been designed to gain insight into the general effect of electrical current on cells specifically regarding such parameters as alteration and growth, and multiplication rates, orientation and metabolic demands (36).

MATERIALS AND METHODS

Primate fibroblasts are being grown in suspension and in monolayer cultures using commercially-available media supplemented with fetal calf serum and antibiotics (37). Control data are established on both the cell line and

and the media. Analytical procedures that are established and being employed include total cell count (38), viability count (39), karyotype (40), and determinations of total protein (41), collagen (42), and nucleic acid (43, 44). Monolayer cultures are grown on a regular 25 mm. x 75 mm. microscope slide in a specially-designed apparatus (Fig. 2). Direct current at the appropriate current density is applied throughout the period required for the monolayer to be completely formed, and subsequently the monolayer is fixed and prepared for cytologic examination.

RESULTS AND DISCUSSION

Cultures receiving 0.1 and 1.0 microampere direct current, pulsed direct current, and alternating current exhibit slightly increased total cell counts when compared to cultures receiving no current. However, there is no significant change in cell viability. Upon microscopic examination of the monolayer culture, one finds an uneven distribution of mitotic figures which are more numerous in the area of the cathode. Cells bordering the anode appear microscopically less vital with pyknotic nuclei and granular cytoplasm, as well as an increased incidence of giant cells and anisokaryosis and cellular pleomorphism.

The above observations are based upon examination of 10 monolayer cultures at each current level (0.1 and 1.0 microampere) using each current type, direct, pulsed direct and alternating.

FIGURE III

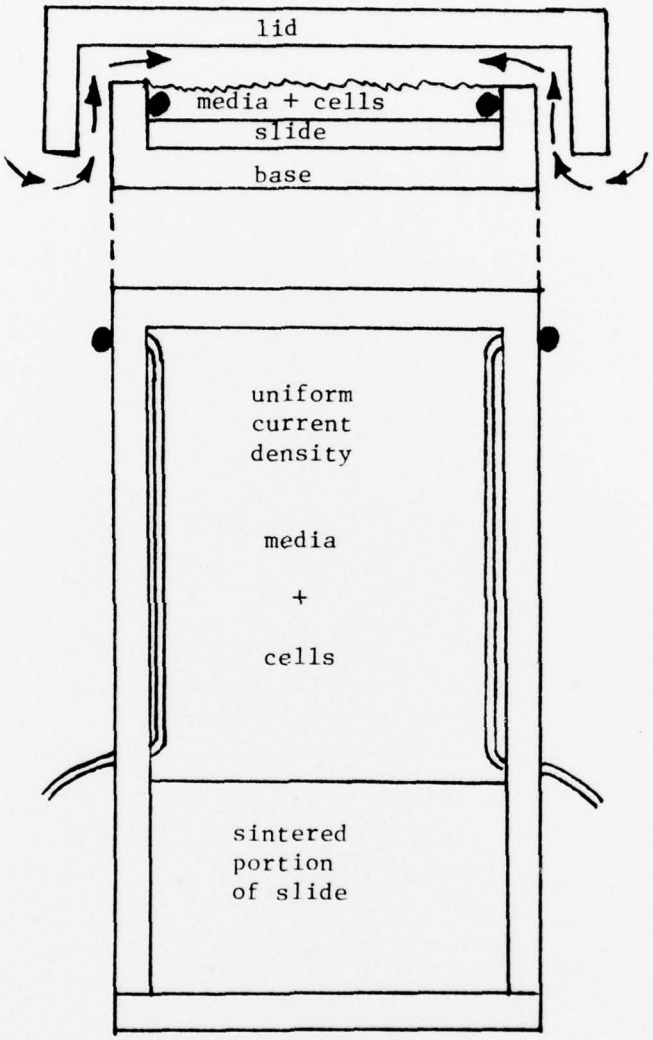


Diagram of the experimental chambers for the administration of electrical currents to cells in culture. Top: side view of chamber and contents, with the electrodes in cross-section indicated as solid circles. Below: top view of chamber indicating the long parallel electrode placement to create a uniform current density.

PROJECT SUMMARY

Work conducted by our laboratory in the study of the electrical enhancement of the reparative process of hard tissue has resulted in the following:

1. The development of a carefully controlled experimental model productive of quantitative data that can be subjected to rigid statistical analysis.

2. Statistically significant data demonstrating the positive effects of the application of an exogenous electric current to the healing wound in hard tissue.

3. Statistically significant data demonstrating the effect of electrode configuration when direct current is administered to a healing wound in hard tissue.

4. Statistically significant data demonstrating differences in the effects of varied current densities when applied to the healing wound in hard tissue.

5. Significant data demonstrating that administration of exogenous current early in the healing process is more efficacious than later administration.

6. Statistically sound data demonstrating no significant effect between the application of pulsed direct current and alternating current at the 1.0 microampere level.

7. Statistically sound data demonstrating no significant effect between the administration of direct and pulsed direct current at the 1.0 microampere level.

8. Valid observations yet to be confirmed by statistical analysis include a) reversal of the effects of polarity at higher current densities during the administration of direct current; b) the cytopathogenic effect of high-level direct current when applied to cell monolayers; c) the production of periosteal hard tissue excrescences in fields of high current density; and d) alternation in rates of bone apposition in sites remote from stimulated areas.

The above are a portion of observations that have been recorded in our laboratory and are listed merely to illustrate the value of this model system in the production of significant data and its versatility in posing important biological questions.

Our present data suggests that the following parameters be examined:

1. Further elaboration of the effects of varied current levels on the reparative process.

2. Further elaboration of the optimum period in the reparative process for current administration.

3. Correlation of the observations made in the calvarium with similar defects in long bones and in the mandible.

4. The application of exogenous electric current to tooth hard tissue following injury.

5. The admininistration of electrical current to experimental fractures in the dog mandible.

6. The administration of electric current to stimulate apposition of alveolar bone in the face of periodontal disease or at least the attenuation of its loss.

Again, these are but a portion of the questions that arise in the consideration of our data, but it would seem that these are of the nature that would lend themselves to rather simple evaluation when studied in a quantifiable system.

LITERATURE CITED

1. Bassett, C. A. L. (1968). Biologic significance of piezoelectricity. Calc. Tis. Res. 1:252.

2. Becker, R. O., Bassett, C. A., and Bachman, C. C. (1964). Bioelectric factors controlling bone structure. In: H. M. Frost, editor, Bone Biodynamics, Little, Brown and Co., Boston, Ch. 13.

3. Cochran, G. V. B., Pawluk, R. J., and Bassett, C. A. L. (1968). Electro-mechanical characteristics of bone under physiologic moisture conditions. Clin. Orthop. 58:249.

4. Shamos, M. H., Lavine, L. S., and Shamos, H. I. (1963). Piezoelectric effect in bone. Nature 197:81.

5. Bassett, C. A. L., and Becker, R. O. (1962). Generation of electric potentials in response to mechanical stress. Science 137:1063.

6. Friedenberg, Z. B., and Brighton, C. T. (1966). Bioelectric potentials in bone. J. Bone Jt. Surg. 48A:915.

7. Bassett, C. A. L. (1966). Electro-mechanical factors regulating bone architecture. In: Fleisch, H., H. Blackwood and M. Owen, editors, Proceedings of the Third European Symposium on Calcified Tissue. Springer-Verlag, New York, p. 78.

8. Levy, D. D. (1971). Induced osteogenesis by electrical stimulation. Ph.D. Thesis, Polytechnic Institute of Brooklyn.

9. Harrington, D. B. and Meyer, R. Jr. (1974). Effects of small amounts of electric current at the cellular level. Ann. N.Y. Acad. Sci. 238: 300-306.

10. Black, J. and Korostoff, E. (1974). Strain-related potentials in living bone. Ann. N.Y. Acad. Sci. 238:95-120.

11. Marino, A. A. and Becker, R. O. (1970). The effect of electric current on rat tail tendon collagen in solution. Calc. Tis. Res. 4:330.

12. Norton, L. A. and Becker, S. J. (1972). Altered growth of a cultured bone in an electric field. IADR Abstracts, No. 602.

13. Wasserman, F. and Yaeger, J. A. (1969). The matrices of mineralizable tissues. Internat. Dent. J. 19:308.

14. Assimacopoulos, D. (1968). Wound healing promotion by the use of negative electric current. The American Surgeon 34(6):423-431.

15. Wolcott, L. E., Wheeler, P. C., Hardwicke, H. M. and Rowley, B. A. (1969). Accelerated healing of skin ulcers by electrotherapy: Preliminary clinical results. Southern Medical Journal 62(7):795-801.

16. Connolly, J. F., Ortiz, J., Price, R. R. and Bayuzick, R. J. (1973). The effect of electrical stimulation on the biophysical properties of healing fractures. The New York Academy of Sciences.

17. Friedenberg, Z. B., Roberts, P. G., Didizian, N. H. and Brighton, C. T. (1971). Stimulation of fracture healing by direct current in the rabbit fibula. The Journal of Bone and Joint Surgery 53-A(7): 1400-1407.

18. Bassett, C. A. L., Pawluk, R. J. and Pilla, A. A. (1973). Augmentation of fracture repair by electromagnetic fields. The New York Academy of Sciences, Conference on Electrically Mediated Growth Mechanisms in Living Systems, Abstracts, No. 17.

19. Yasuda, I. and Noguchi, K. (1955). Dynamic callus and electric callus. J. Bone Jt. Surg. 37A:1292.

20. Becker, R. O. (1961). The bioelectric factors in amphibian-limb regeneration. J. Bone Jt. Surg. 43-A:643.

21. Friedenberg, Z. B. and Kohanim, M. (1968). The effect of direct current on bone. Surg. Gynec. Obstet. 127:97.

22. Becker, R. O. and Murray, D. G. (1967). A method for producing cellular dedifferentiation by means of very small electrical currents. Trans. N.Y. Acad. Sci. 29:606.

23. Bassett, C. A. L. and Becker, R. O. (1962). Generation of electric potentials by bone in response to mechanical stress. Science 137:1063.

24. Shamos, M. H. and Lavine, L. S. (1965). Bioelectric effects in tissue. Clin. Orthop. 43:254.

25. Yasuda, I., Noguchi, K. and Sata, T. (1955). Dynamic callus and electric callus. J. Bone Jt. Surg. 37-A:1292.

26. Lavine, L. S., Lustrin, I., Shamos, M. H. and Moss, M. L. (1971). The influence of electric current on bone regeneration in vivo. Acta Orthop. Scandinav. 42:305.

27. Bartley, M. H., Arnold, J. S., Haslam, R. K. and Jee, W. S. S. (1966). The relationship of bone strength and bone quantity in health. Disease and Aging, Journal of Gerodontology 21(4):517-522.

28. Weaver, J. K. and Chalmers, J. (1966). Cancellous bone: Its strength and changes with aging and an evaluation of some methods for measuring its mineral content. J. Bone Joint Surg. (Amer.) 48:289-298.

29. The Upjohn Company, Kalamazoo, Michigan.

30. Tetracyn (tetracycline hydrochloride) Pfizer, Inc., New York.

31. Miller, R. A., Rall, D. P. and Toby, J. E. (1957). Bone localization of the tetracyclines. J. Nat. Cancer Inst. 19:87.

32. Savara, B. S., Fields, R. W. and Tacke, R. B. (1973). Electrical modification of tissue architecture in human bone. In press.

33. Levenson, S. N., et. al. (1965). The healing of rat skin wounds. Ann. Surg. 161:293.

34. Madden, J. W. and Peacock, E. E. (1968). Studies on the biology of collagen during wound healing. I. Rate of collagen synthesis deposition in cutaneous wounds of the rat. Surgery 64:288.

35. Ross, R. (1969). Wound healing. Scient. Amer. 220:40.

36. There is a preponderance of literature published on this topic. However, we are unable to use the published data as it applies to neither our specific cell line nor to our techniques of culture.

37. Microbiological Associates Co., Bethesda, Maryland.

38. Paul, J. (1970). Cell and Tissue Culture, fourth edition.

39. Merchant, D. J., Raymond, H. K. and William, M. H. (1964). Handbook of Cell and Organ Culture. J. R. Burgess Co., Minneapolis, p. 120.

40. Hecht, F., Wyandt, H. E. and Magenis, R. E. (in press). The human cell nucleus; quinoquin and other differential stains of chromatin and chromosomes. In: H. Busch, editor, The Cell Nucleus, Academic Press.

41. Kabat, E. A. and Mayer, M. M., editors (1948). Experimental Amino Chemistry. Charles C. Thomas, Springfield, Ill., Ch. 22.

42. Stanton, G. and Levy, M. (1969). Method for the correlation of chemical and histological composition. J. Dent. Res. 48:38-42.

43. Paul, J. (1956). A simple method for the determination of DNA in tissue culture. J. Biophys. Biochem. Cytology. 2:797.

44. Paul, J. (1958). Determination of the major constituents of small amounts of tissue. Analyst. 83:37.

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